Disease characteristics of MCT8 deficiency: an international, retrospective, multicentre cohort study

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Abstract: Background: Disordered thyroid hormone transport, due to mutations in the SLC16A2 gene encoding monocarboxylate transporter 8 (MCT8), is characterised by intellectual and motor disability resulting from cerebral hypothyroidism and chronic peripheral thyrotoxicosis. We sought to systematically assess the phenotypic characteristics and natural history of patients with MCT8 deficiency. Methods: We did an international, multicentre, cohort study, analysing retrospective data from Jan 1, 2003, to Dec 31, 2019, from patients with MCT8 deficiency followed up in 47 hospitals in 22 countries globally. The key inclusion criterion was genetically confirmed MCT8 deficiency. There were no exclusion criteria. Our primary objective was to analyse the overall survival of patients with MCT8 deficiency and document causes of death. We also compared survival between patients who did or did not attain full head control by age 1 · 5 years and between patients who were or were not underweight by age 1-3 years (defined as a bodyweight-for-age Z score <-2 SDs or <5th percentile according to WHO definition). Other objectives were to assess neurocognitive function and outcomes, and clinical parameters including anthropometric characteristics, biochemical markers, and neuroimaging findings. Findings: Between Oct 14, 2014, and Jan 17, 2020, we enrolled 151 patients with 73 different MCT8 (SLC16A2) mutations. Median age at diagnosis was 24 · 0 months (IQR 12 · 0-60 · 0, range 0 · 0-744 · 0). 32 (21%) of 151 patients died; the main causes of mortality in these patients were pulmonary infection (six [19%]) and sudden death (six [19%]). Median overall survival was 35 · 0 years (95% CI 8 · 3-61 · 7). Individuals who did not attain head control by age 1 · 5 years had an increased risk of death compared with patients who did attain head control (hazard ratio [HR] 3 · 46, 95% CI 1 · 76-8 · 34; log-rank test p=0 · 0041). Patients who were underweight during age 1-3 years had an increased risk for death compared with patients who were of normal bodyweight at this age (HR 4 · 71, 95% CI 1 · 26-17 · 58, p=0 · 021). The few motor and cognitive abilities of patients did not improve with age, as evidenced by the absence of significant correlations between biological age and scores on the Gross Motor Function Measure-88 and Bayley Scales of Infant Development III. Tri-iodothyronine concentrations were above the age-specific upper limit in 96 (95%) of 101 patients and free thyroxine concentrations were below the age-specific lower limit in 94 (89%) of 106 patients. 59 (71%) of 83 patients were underweight. 25 (53%) of 47 patients had elevated systolic blood pressure above the 90th percentile, 34 (76%) of 45 patients had premature atrial contractions, and 20 (31%) of 64 had resting tachycardia. The most consistent MRI finding was a global delay in myelination, which occurred in 13 (100%) of 13 patients. Interpretation: Our description of characteristics of MCT8 deficiency in a large patient cohort reveals poor survival with a high prevalence of treatable underlying risk factors, and provides knowledge that might inform clinical management and future evaluation of therapies. Funding: Netherlands Organisation for Health Research and Development, and the Sherman Foundation.

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Abstract:	Background: Disordered thyroid hormone transport, due to mutations in monocarboxylate transporter 8 (MCT8; gene: SLC16A2), is characterized by intellectual and motor disability due to cerebral hypothyroidism and chronic peripheral thyrotoxicosis. Phenotypic characteristics and natural history of MCT8 deficiency have not been systematically evaluated. Methods: In this international, multicentre, study, retrospective data (2003 to 2019) from patients with MCT8 deficiency followed in 47 centres, was analysed. Our primary objectives were to determine neurocognitive outcomes and overall survival. We also assessed clinical parameters, including anthropometric characteristics, biochemical markers and neuroimaging findings. Results: 151 subjects with 73 different MCT8 (SLC16A2) mutations were included. 21·2% (32/151) of patients died, with main causes of mortality in these patients being pulmonary infection (18·8%) and sudden death (18·8%). The median overall survival was 35·0 (95%CI 8·3-61·7) years. Survival differed significantly between individuals who attained head control by the age of 1·5 years or not (log-rank test: p=0·0041; hazard ratio 3·46 95%CI 1·76-8·34). Patients who were underweight during early childhood (1-3 years of age) had an increased risk for death compared with patients who were not underweight at this age (HR 4·71, 95% CI 1·26-17·58, p=0·021). The limited motor and cognitive abilities of patients did not improve with age. T3 concentrations were elevated in 95·1% (96/101) and total T4 concentrations were reduced in 89·5% (94/105) of patients. 71·1% (59/83) patients were underweight (<-2SD). Cardiovascular abnormalities were frequent, with 53·2% (25/47) of patients exhibiting elevated systolic blood pressure, and 75·6% (34/45) of patients having premature atrial contractions and 31·3% (20/60) having resting tachycardia. Interpretation Our description of characteristics of MCT8 deficiency in a large patient cohort reveals poor survival with a high prevalence of treatable underlying risk factors and provides knowledge which informs clinical management and future evaluation of therapies. Funding Our study was funded by the Netherlands Organisation for Health Research and Development (project number 113303005; to WEV), and the Sherman Foundation (to WEV).

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Abstract

Background: Disordered thyroid hormone transport, due to mutations in monocarboxylate transporter 8 (MCT8; gene: *SLC16A2*), is characterized by intellectual and motor disability due to cerebral hypothyroidism and chronic peripheral thyrotoxicosis. Phenotypic characteristics and natural history of MCT8 deficiency have not been systematically evaluated.

Methods: In this international, multicentre, study, retrospective data (2003 to 2019) from patients with MCT8 deficiency followed in 47 centres, was analysed. Our primary objectives were to determine neurocognitive outcomes and overall survival. We also assessed clinical parameters, including anthropometric characteristics, biochemical markers and neuroimaging findings.

Results: 151 subjects with 73 different MCT8 (*SLC16A2*) mutations were included. 21·2% (32/151) of patients died, with main causes of mortality in these patients being pulmonary infection (18·8%) and sudden death (18·8%). The median overall survival was 35·0 (95%CI 8·3-61·7) years. Survival differed significantly between individuals who attained head control by the age of 1·5 years or not (log-rank test: p=0·0041; hazard ratio 3·46 95%CI 1·76-8·34). Patients who were underweight during early childhood (1-3 years of age) had an increased risk for death compared with patients who were not underweight at this age (HR 4·71, 95% CI 1·26-17·58, p=0·021). The limited motor and cognitive abilities of patients did not improve with age. T3 concentrations were elevated in 95·1% (96/101) and total T4 concentrations were reduced in 89·5% (94/105) of patients. 71·1% (59/83) patients were underweight (<-2SD). Cardiovascular abnormalities were frequent, with 53·2% (25/47) of patients exhibiting elevated systolic blood pressure, and 75·6% (34/45) of patients having premature atrial contractions and 31·3% (20/60) having resting tachycardia.

Interpretation Our description of characteristics of MCT8 deficiency in a large patient cohort reveals poor survival with a high prevalence of treatable underlying risk factors and provides knowledge which informs clinical management and future evaluation of therapies.

Funding Our study was funded by the Netherlands Organisation for Health Research and Development (project number 113303005; to WEV), and the Sherman Foundation (to WEV).

Research in context

Evidence before this study

Monocarboxylate transporter 8 (MCT8) deficiency is a rare genetic disorder with devastating consequences including intellectual and motor disability due to cerebral hypothyroidism and severe clinical sequelae secondary to chronic peripheral thyrotoxicosis. We searched Pubmed for studies published in English to January 1, 2020, using the search terms “MCT8 deficiency”, “Allan-Herndon-Dudley Syndrome”, “AHDS”, “natural history” and “life expectancy”. Prior to this study, given the rarity of the disorder, knowledge on the phenotypic characteristics, natural history and life expectancy of monocarboxylate transporter 8 (MCT8) deficiency was limited. Previous studies consisted of case reports, had small patient cohorts (<25 patients), and neglected the peripheral features of the disorder. Comprehensive and structured in-depth characterisation of the phenotype of MCT8 deficiency is urgently needed to accelerate early diagnosis and inform management, including the use of a new disease-modifying therapy.

Added value of this study

151 patients from 47 centres across the world were included in the largest study on MCT8 deficiency, to our knowledge. This is the first multicentre, international study to provide in-depth quantitative data on the natural history and life expectancy of patients with MCT8 deficiency. Our data report poor survival in this disorder, with 30% of patients dying in childhood. Having identified pulmonary infection and sudden death (our data suggests cardiac arrhythmia as underlying basis) as the major causes of mortality, timely intervention with Triac

therapy may ameliorate the poor prognosis in this disease. Also, the identification of underweight being strongly linked to survival provides a direct target for clinical management. Our detailed description of key clinical features together with biochemical and radiological correlates constitutes a signature for the disorder which may facilitate its early diagnosis and discrimination of this entity from other developmental disorders. Our data will be used as natural history control data for an ongoing trial of with Triac in young children with MCT8 deficiency (NCT02396459). These data will also be important for future clinical trials investigating treatment options for MCT8 deficiency, such as gene therapy.

Implications of all the available evidence

Systemic in-depth description of international natural history data will inform clinical management of patients with MCT8 deficiency. Our findings underscore the need for a multidisciplinary approach in the management and follow-up of patients with MCT8 deficiency. The current data indicate a unique combination of clinical presentation, biochemical markers and brain imaging features that will enhance early diagnosis. The low T4 concentrations measured in the neonatal screening indicates that current neonatal screening strategy holds potential to detect MCT8 deficiency. These observations hinting at the possibility of early diagnosis are particularly relevant in the context of Triac therapy recently reported, which has the potential to ameliorate the devastating course of the disorder if left untreated. In addition, robust natural history data can be used as controls in clinical trials for rare diseases in which accrual of placebo controls in group might not be feasible.

Introduction

Thyroid hormones are crucial for normal physiological processes, particularly neurodevelopment, and regulation of basal metabolic rate, throughout life (1, 2). Intracellular bioavailability of thyroid hormones is governed by membrane transporter proteins that facilitate their cellular entry (3). Monocarboxylate transporter 8 (MCT8) is a specific thyroid hormone transporter that is crucial for transport of triiodothyronine (T3) and thyroxine (T4) in several tissues, including the brain (4-8). Mutations in the gene encoding MCT8 (*SLC16A2* on chromosome Xq13.2) cause MCT8 deficiency, also known as Allan-Herndon-Dudley syndrome (AHDS), a debilitating disorder with an estimated prevalence of 1 in 70 000 male individuals (9-11).

MCT8 deficiency is characterized by profound neurodevelopmental delay and a wide range of severe clinical sequelae secondary to chronic peripheral thyrotoxicosis which cannot be effectively treated with conventional (anti)thyroid drugs (3, 10, 11). In 2019, a clinical trial showed that treatment with triiodothyroacetic acid (Triac) ameliorates key features of peripheral thyrotoxicosis and might improve neurocognitive outcomes if treatment is commenced early in life (12).

Robust, comprehensive data regarding the phenotypic characteristics and natural history of patients with MCT8 deficiency are lacking, as the phenotype has only been recorded in single case reports or small case series with related patients [*e.g.* (13, 14)]. Furthermore, these reports used differing clinical methods precluding consistent assessments, and merely focused on the neurological phenotype, neglecting the peripheral clinical features of the disorder (3, 14). Data on survival and neurodevelopmental outcomes in this disorder are not known. The lack of consistent quantitative knowledge of the natural history and the

phenotypic spectrum of MCT8 deficiency hampers early diagnosis and uniform clinical management including the evaluation of a disease-modifying therapy.

Given the paucity of recorded data and with access to a large patient cohort via an international collaborative network for this rare disorder, we have sought to provide comprehensive and uniform phenotypic characterization of MCT8 deficiency using clinical, radiological, and biochemical data.

Methods

Study design and participants

This international study was initiated on 14 October 2014 by founding a consortium of centres where patients with MCT8 deficiency were followed before and after this date.

The key inclusion criterion was genetically confirmed MCT8 deficiency. Additionally, data on first-degree and second-degree male relatives with clinical MCT8 deficiency (when genetic testing was not available at that time) were included. There were no exclusion criteria. Our cohort consisted of patients, evaluated with a standardized protocol, who had been enrolled in the international, multicentre Triac Trial [NCT02060474, (12)] and patients who participated in the named patient program for Triac treatment and historical cases for whom Erasmus MC fulfilled a consultancy role following the first reports of MCT8 deficiency in 2004 (10, 11) (**figure s1**). The group of historical cases therefore contain patients who were alive and patients who were already deceased at time of enrollment. A subgroup of participants has been reported before with available individual case descriptions (n=47), or has been reported on aggregated level (n=46, (12)) (**figure s2**). For such patients, updated and exhaustive data were collected. For analysis of serum thyroid function tests, only patients

whose measurements were performed in the central laboratory of the Erasmus MC were considered to avoid inter-assay variation. For in-depth clinical and biochemical phenotyping only those patients were enrolled that either participated in the Triac Trial (12) or in the named patient program to ensure data had been captured by trained personnel and according to standard operating procedures.

Ethical considerations

This study conforms to the Declaration of Helsinki, Good Clinical Practice guidelines and was evaluated and approved by the appropriate local institutional review boards or ethics committees. However, for the retrospective analysis of existing datasets of patients in routine clinical care, the majority of centres did not require additional specific institutional review board approval. For other centres, studies were either ethically approved or the ethics committee provided a waiver for approval. Informed consent was obtained from the parents or legal representatives of all enrolled patients, unless the relevant institutional review board and/or local regulations had authorized the use of anonymised patient data without additional consent.

Procedures

An overview of study assessments and investigations is provided in **figure s1** and in the **Supplementary Methods**.

Outcomes

Our primary objective was to analyse the overall survival of patients with MCT8 deficiency and document causes of death. We also compared survival between patients who

did or did not attain full head control by the age of 1·5 years and between patients who were or were not already underweight by early childhood (between 1-3 years of age).

Other key objectives were to document neurocognitive function using uniform criteria and assess their relationship to biological age as a measure of disease progression and developmental outcome and to describe the occurrence of extra-neurological features.

Statistical analysis

We summarised continuous variables as mean and standard deviation (SD), or median and range. We established overall survival and compared patients with and without full head control and cases who were or were not underweight during early childhood with log-rank analysis. Survival was defined as the age at last date known alive. Hazard ratios were calculated using Cox regression models. Correlations between biological age and scores on different neuropsychological assessments were explored using linear regression. For these analyses, we excluded patients with a less severe neurocognitive phenotype, defined as individuals that attained at least two of the following developmental milestones: talking in simple words, achieving head control, sitting independently, and/or walking with assistance. Higher developmental attainment in these patients is more likely to be due to the milder impact of the underlying MCT8 mutation than to the effect of aging. Assumptions for linear regression analyses were met. All statistical tests were two-sided, and p values of less than 0·05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism, version 6 (GraphPad, La Jolla, CA, USA).

Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results

In 47 centres, 151 patients of 22 different nationalities (8 ethnicities) were enrolled between October 14th 2014 and January 17th 2020 (**figure s1**), thereby including 50 percent of families reported thus far (**figure s2**). In 106 cases serum thyroid function tests had been measured in the central laboratory, and 86 had been checked according to standardised protocols for in-depth phenotyping at a median age of 4·8 years (interquartile range [IQR] 1·9-9·8, range 0·4-66·8) (**figure s1**).

The demographics and characteristics of the enrolled patients are summarised in **table s1**. In the 151 enrolled cases, 73 different underlying MCT8 mutations were identified, of which 36 had not been reported before (**figure s3**). A total of 17 mutations were identified in at least two unrelated families. All 35 missense mutations were located in the transmembrane helices (**figure s4**). The median age at diagnosis was 24·0 months (range: 0·0-744·0) (**figure 1A**), but the median age at onset of first symptoms was 4·0 months (range: 0·0-13·0) (**figure 1A**). Consequently, the median time to diagnosis was 18·0 months (IQR 7·8-63·0, range 0·0-738·0). The most frequently reported initial concerns that prompted medical evaluation were gross developmental delay (78·6%), hypotonia (39·8%), feeding problems (8·2%), and poor weight gain (7·1%) (**figure 1B**).

32 (21·2%) patients had died and the median age of their demise was 10·5 years (IQR 5·3-18·8, range 1·6-71·0). The main causes of death reported for these patients were

pulmonary infections (18·8%), sudden death (18·8%), and aspiration pneumonia (9·4%) (**figure 1C**). In 15 (46·9 %) of 32 deceased subjects the cause of death was unclear and postmortem examinations had not been performed. The median overall survival was 35·0 years (95% CI 8·3-61·7; **figure 1D**). The 10-, 18-, and 60-year survival probabilities were 85% (95% CI 78·0-92·0), 69·8% (58·2-80·3), and 34·8% (10·2-59·3), respectively. Patients not attaining full head control by the age of 1·5 years had an increased risk for death compared with patients who did attain head control (HR 3·46, 95% CI 1·76-8·34, p=0·0041; **figure 1E**). Patients who were underweight during early childhood (1-3 years of age) had an increased risk for death compared with patients who had a normal body weight by this age (HR 4·71, 95% CI 1·26-17·58, p=0·021, **figure 1F**).

The prevalence of specific neurological features in patients included in the in-depth phenotyped cohort (N=86, median age 4·8 years, IQR 1·9-9·8, range 0·4-66·8) is reported in **table 1** and **figure 2**, and neurological sequelae are summarized in **figure s5** and **figure s6**. All patients had moderate-to-severe intellectual disability with a severe delay in motor and language development (**table s2**). Only 6 (7·7%) patients achieved independent sitting and were less severely affected than the other patients (**figure 2A**). The median score on the Gross Motor Function Measure (GMFM)-88 (15) did not exceed 10% of the total score that should be obtained by healthy 4-year old children (**figure 2B**, **table s2**). Among 28 subjects that had been evaluated at a median age of 6·4 years (range 0·4-44·6) with the Bayley Scales of Infant Development (BSID)-III (16), the median developmental age was well-below 12 months on all tested sub-domains (**figure 2C-G**, **figure s7B-F**, **table s2**). Similar findings were obtained with the Vineland Adaptive Behavior Scale (VABS)-II (**figure s8, table s2).** The scores in any of the developmental domains did not correlate positively with age (e.g. motor skills: GMFM-88 B=-0·10 (95% CI, -0·29-0·09; p=0.29), BSID-III fine motor skills B= -0·11 (-0·23 - 0·01; p=0.072), and

BSID-III gross motor skills B= -0·04(-0·11- 0·02; p=0.17); **figure 2C-G**) and scores of patients with different age categories were not different (**table s2**)).

Pregnancy and delivery were unremarkable in the majority of cases, with most infants having normal birth weight and head circumference (**table 1**). At first presentation, most patients had global hypotonia with a pronounced head-lag on vertical suspension and upper trunk slipping through. Typically, by the end of the first year, dystonic posturing of the limbs and neck were noted. Exaggerated deep tendon reflexes were present in 80·3% (57/71) of cases, and 90·5% (67/74) of patients developed hypertonia in wrists, knees or heels with age attributed to dystonia and spasticity. Primitive reflexes remained present in 91·1% (51/56) cases, with a positive tonic neck reflex (81·0%) and glabellar sign (80·0%) being most prevalent, irrespective of patient age. Electro-encephalogram (EEG)-confirmed seizures were observed in 15 (23·1%) of 65 patients, and mostly involved generalized, absence-like episodes without a clear motor component.

MRI scans of the brain were available in 13 patients, performed at a median age of 8·0 months (range: 5·0-187·0), with 8 patients having at least one follow-up scan available (**table 1, figure s5**, **table s3**). The most consistent finding was a global delay in myelination, evidenced by diffuse residual hyperintense white matter in specific brain regions on T2-weighted images. Myelination improved with age, but had not fully normalized in the oldest patient (15 years) with available data. The neuroradiological findings were supported by postmortem findings (see **supplementary results**).

Serum thyroid function tests were available in 106 treatment-naïve patients at a median age of 5·3 years (IQR 2·1-11·0, range 0·4-66·8). Serum TSH concentrations were within the normal range in 93 (88·6 %) of 105 patients (**figure 3A**). Serum free and total T4

concentrations were below the age-specific lower limit in 94 (88·7 %) of 106 and 94 (89·5%) of 105 patients, respectively (**figure 3B** and **figure s9A**). Mean serum T3 concentrations exceeded the age-specific upper limit in 96 (95·1%) of 101 patients (**figure 3C**), which resulted in a pronounced increase in the T3/T4 ratio (**figure s9B**). Reverse T3 (rT3) concentrations were decreased in 76 (90·5%) of 84 patients (**figure s9C**), with a concomitant increase in the T3/rT3 ratio (**figure s9D**). This endocrine signature was present regardless of age (**figure s9E-H**). In 3 out of 7 subjects TRH-stimulation tests showed an inadequate TSH response. In 7 (87·5%) out of 8 subjects in whom T4-based neonatal screening results were available, total T4 concentrations were below the 20th percentile (**figure 3D**), and in 5 out of 8 (60%) below the 10th percentile. By contrast, neonatal TSH concentrations were <15 mU/L in 8/8 (100%) of patients with available data (**figure s9I**). Serum total T4 concentrations were significantly less reduced in patients with less severe versus those with a severe neurocognitive phenotype (1·05±0·22 vs 0·71±0·18 times the age-specific lower limit of normal, p<0·0001) (**figure s10A)**. Serum T3 concentrations were not significantly different between these groups (1·46±0·23 vs 1·51±0·44 times the age-specific upper limit of normal, p=0·76)(**figure s10B**). Consequently, the T3/T4 ratio, a marker of thyroid hormone metabolism in peripheral tissues, was significantly lower in patients with a less severe phenotype (1·44±0·40 vs 2·27±0·91 times the age-specific upper limit of normal, p=0·019) (**figure s10C**).

The main findings of in-depth phenotyping of peripheral clinical features (n=86) are summarised in **table 2** and **table s4**. Body weight for age showed progressive deterioration over time, with 59 (71·1%) of 83 patients being severely underweight (<-2SD) (**figure 3E, table 2**). 27 (35·5%) of 76 patients were tube fed, although impaired swallowing function was reported in 55 (71·4%) of 77 patients. Body height for age also deteriorated with age (**figure 3F**). Delayed sexual maturation was present in 5 (26·3%) patients (**figure s11A-D**). Among

patients over 8 years of age, bone mineral density (BMD) was below the 5th percentile, but bone turnover markers were generally within the low-normal range (**figure s12A-D**).

The mean resting heart rate was 110 (±20) beats per minute, with 20 (31·3%) of 64 patients exceeding the 90th percentile for age (**figure 3G**) (17). Systolic blood pressure exceeded the 90th percentile in 25 (53·2%) of 47 patients whereas the diastolic blood pressure exceeded the 90th percentile in 17 (36·2%) of 47 patients (**figure 3H**) (18, 19). Detailed cardiovascular assessment was available in 50 patients. At the time of evaluation, 47 (94%) of 50 patients reportedly had no cardiovascular abnormalities and were not receiving any treatment. 3 (6·0%) of 50 patients had second-degree atrioventricular block (Mobitz I: 1; Mobitz II: 2) and 6 (12·0%) of 50 patients had (incomplete) right bundle branch block. In addition, corrected QT interval (QTc) was above the 98th percentile in three out of 39 (7·7%) patients (**table 2**, **figure s13A**). Even though most patients were completely immobile, 24h ambulatory cardiac monitoring showed a high resting heart rate (103±13 beats per minute) with frequent episodes of tachycardia and premature atrial or ventricular contractions (**figure 3I, table s4**). One childhood patient had an episode of atrial fibrillation and another had non-sustained ventricular tachycardia (**table 2**). Cardiac echocardiography studies performed in 26 patients revealed dilated aortic root (>+2SD for age, range 2·0-3·4 SD) in 7 (26·9%) patients, and relatively small left ventricular wall dimensions (**figure s13B**).

Serum concentrations of sex hormone binding globulin were elevated in 69 (88·5%) of 78 patients (**figure 3J, figure s14**). Serum alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase concentrations were mildly elevated in a substantial proportion of patients (**table 2, figure s15D-F**), and two patients reportedly had an episode of hepatic dysfunction following a (viral) infection.

43 (84·3%) of 51 patients had low muscle mass. Creatinine concentrations in serum were within the low-normal range for age (**figure 3K, s14B**). Serum creatine kinase concentrations were mostly low-normal (**figure 3L, s14C**), with some exceptions in patients with recent seizures or severe dystonic episodes. Other biochemical tests are shown in table s4 and figure s15A-O.

Gastroesophageal reflux disease was present in 79·2% (38/48) of patients and this often required pharmacological intervention. Spontaneous gastrointestinal bleeding was reported in 2 patients and was the cause of death in one of them. Constipation was present in 62·3% (37/63) of patients. 29 (69·1%) of 42 patients had recurrent (pulmonary) infections (**table 2**).

Discussion

To our knowledge, this international, multicentre, retrospective study reports the quantitative evaluation of the disease characteristics of MCT8 deficiency, in the largest cohort of patients with this disorder. We have documented key clinical features together with biochemical and radiological correlates as well as outcomes in this rare but potentially treatable condition. Our findings will facilitate accurate diagnosis, guide management, and inform conduct of future therapeutic intervention trials.

A principal finding is that overall survival of patients with MCT8 deficiency is greatly diminished, with an overall median life expectancy of 35 years. Stratification of analyses revealed that patients who attain full head control are more likely to survive longer than those who do not. Accordingly, attaining full head control, as a marker of improved

298 neurodevelopment, could be a relevant endpoint for future therapeutic trials in MCT8
299 deficiency.

300 The most common cause of death was pneumonia, caused either by aspiration or by
301 infections. Aspiration, due to impaired swallowing function, is frequently observed in patients
302 with MCT8 deficiency, and could be mitigated by tube feeding. However, a substantial number
303 of patients that exhibited swallowing problems were not tube fed and thus remained at risk
304 for aspiration. With our study suggesting that being underweight is strongly linked to reduced
305 survival, tube feeding can prevent adverse clinical sequelae and potentially improve life
306 expectancy (20). The second major cause of mortality was sudden death. Although its
307 aetiology remains unclear, available data may suggest a cardiac cause, with the high
308 prevalence of premature atrial and ventricular contractions, which are uncommon in healthy
309 individuals especially in childhood (21-25). We also observed non-sustained ventricular
310 tachycardia and QTc prolongation in some patients, with both considered risk factors for
311 sudden cardiac death. Moreover, a substantial proportion of patients exhibited systolic
312 hypertension and/or tachycardia and had several echocardiographic and electrophysiological
313 cardiac changes that have been linked to these traits. As the vast majority (94·0%) of patients
314 reportedly had no history of cardiac problems, these cardiovascular abnormalities likely
315 remain clinically undiagnosed in this population. This observation calls for inclusion of
316 cardiovascular assessment in the management of this disorder. With loss of body weight and
317 many cardiovascular abnormalities being attributable to chronic thyrotoxicosis, reduction in
318 circulating T3 concentrations in patients could represent effective treatment for these aspects
319 of the disorder. Indeed, in a recent clinical trial, treatment with the thyroid hormone analogue
320 Triac efficiently reduced serum T3 concentrations and improved key clinical features such as

loss of body weight and reversal of abnormal cardiovascular parameters in MCT8 deficiency (12).

The current study also identified several other clinical features that require treatment or close follow-up, of which gastro-esophageal reflux disease, scoliosis, hip luxation, and constipation have the highest prevalence. The presence of mildly elevated aminotransferases and the occurrence of transient hepatic failure in at least three reported cases following a viral infection [this report and (11)], suggests that drugs with hepatotoxic side effects (e.g. anti-epileptic drugs as frequently used in this population) should be used with extra caution.

Our comprehensive documentation of neurological sequelae in patients with MCT8 deficiency revealed that the combination of global hypotonia, hypertonia due to dystonia and spasticity and persistence of primitive reflexes was present in up to 90% of patients. Delayed myelination on MRI was consistent with other studies (14, 26, 27). Taken together, these clinical and neuroimaging characteristics may facilitate early diagnosis of MCT8 deficiency and in discriminating this entity from other neurodevelopmental disorders.

Our study highlights major delay in diagnosis of this disorder, with a minority of cases being identified in the first year of life. This is mainly attributable to the non-specific initial clinical features with lack of awareness of the specific characteristics of this disorder among clinicians. Having documented that circulating T3 concentrations are elevated in patients below one year of age, the combination of clinical and radiological features with measurement of serum T3 concentrations may constitute a key clue for early diagnosis. The low T4 concentrations measured in patients with MCT8 deficiency in the neonatal screening indicates the potential to diagnose MCT8 deficiency in newborns. This may engender debate on whether modification of the current neonatal screening strategy is warranted. The importance

of early diagnosis is supported by preclinical studies in which Triac completely prevented abnormal neurological development in animal models of MCT8 deficiency when administrated at birth (28). A future phase 2 clinical trial will investigate the effects of Triac on neurodevelopment, with treatment being commenced at a very young age (NCT02396459).

This study has limitations inherent to its retrospective design. In general, such study design is prone to collection of an incomplete dataset, possibly resulting in selection bias. Indeed, most assessments could not be carried out in all patients, resulting in missing data. The cause of this mostly reflected disease characteristics, such as a poor clinical condition of patients, their inability to follow instructions and dystonic posturing that hampered investigations for which patients needed proper positioning. Therefore, some conclusions are based on a limited number of observations. Yet, calculated prevalence rates for many clinical features were similar to those observed in smaller cohort studies and through analysis of existing literature (14). It was also not possible to obtain uniform long-term follow-up data. However, MCT8 deficiency is a rare disorder with surviving patients being located throughout the world such that retrospective analysis of available clinical data was the most suitable way of increasing our understanding of this disorder. In the majority of cases data had been collected uniformly during baseline assessment of patients whether participating in the Triac Trial or in named patient treatment programs, providing an unique opportunity for systematic cross-sectional evaluation of key clinical outcomes. Should MCT8 deficiency result in an increased rate of miscarriage this will likely remain unascertained, resulting in survivor bias. Although selection bias cannot be excluded, probably because not all newly diagnosed cases are brought to our attention and patients who die before the diagnosis has been established are being missed, our study included a substantial proportion of currently diagnosed patients.

With advent of Triac as possible disease-modifying therapy, a future, prospective cohort study of the natural history of the disorder is unlikely to be possible.

In summary, this study provides a comprehensive and structured in-depth characterisation of the phenotype of MCT8 deficiency. The current study first reports poor survival in this disorder, with 30% of patients dying in childhood. Having identified pulmonary infection and sudden death (our data suggests cardiac arrhythmia as underlying basis) as the major causes of mortality, timely intervention with Triac therapy may ameliorate the poor prognosis in this disease. Furthermore, our finding that survival is particularly poor in patients with impaired neurological development (head control) or who are underweight, provides a basis for therapeutic intervention targeted at this subgroup. Our findings underscore the need for a multidisciplinary approach in the management and follow-up of patients with MCT8 deficiency. In addition, our observations represent an unique, quantitative dataset of the characteristics of this disorder which may serve as a historical control for future interventional studies in this rare disorder, for which a biological control group is often deemed not feasible. Accordingly, we suggest that this study enhances our understanding of the clinical sequelae and longterm outcome of MCT8 deficiency and also facilitates the diagnosis and management of this disorder.

Contributors

SG, FSvG, WEV, IFMdC, and MD designed the study, acquired and analysed the results and drafted and approved the manuscript. All other authors contributed to the acquisition, analysis, and interpretation of data, and approved the manuscript.

Declaration of Interest

WEV reports grants from Netherlands Organisation for Health Research and Development and from Sherman Foundation during the conduct of the study. WEV, SG, FSvG report other from Rare Thyroid Therapeutics, outside the submitted work. DC reports grants from BioMarine, grants from UCB, grants from A&D pharma, outside the submitted work. MCYdW reports other from Hoffmann-La Roche Ltd, other from Ionis, outside the submitted work.. All other authors declare no competing interests.

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Data sharing

Because of the rarity of MCT8 deficiency, individual participant data beyond that reported here will not be shared, to safeguard patient privacy.

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Legends to the Figures

Figure 1 – Panel **A** graphically presents the mean ± SEM (black lines) age at onset of symptoms and the age at time of diagnosis. Grey dots represent measurements in individual patients. Panel **B** shows the most commonly reported features that prompted parents to seek medical attention. Panel **C** shows the causes of death in patient with MCT8 deficiency based on the available information derived from the 32 patients in our cohort who died at a median age of 10·5 years (IQR 5·3-18·8, range 1·6-71·0). HR: hazard ratio, calculated using Cox regression models. Panel **D** shows the overall survival based on age at last follow-up (Kaplan-Meier estimates). Panel **E** shows the Kaplan-Meier estimates of MCT8-specific survival in patients who attained head control (red line) by the age of 1·5 years *versus* those who did not (blue line) and panel **F** those in patients with underweight (blue line) *versus* normal body weight (red line) in early childhood (1-3 years of age). Underweight was defined as a body weight for age z score <-2SDs (or below the fifth percentile), following the definition of the World Health Organization. Please note, since potential confounding factors could not be accounted for, a causal relationship on survival cannot be inferred from the applied stratification variables.

Figure 2 – Panel **A** shows the prevalence of clinical, radiological and developmental key features in MCT8 deficiency. Bars indicate the proportion of patients presenting the indicated feature at first presentation. Panel **B** represents the gross motor function development in patients with MCT8 deficiency measured by the Gross Motor Function Measure (GMFM)-88 (15). A 100% score indicates the level of development that is achieved by a healthy 4-year old child. Panel **C** shows cognition, panel **D** receptive language, panel **E** expressive language, panel **F** fine motor skills and panel **G** gross motor skills, measured by the respective sub-domains of the Bayley Scales of Infant Development (BSID)-III (16). Scores are expressed as developmental age in months. In panels **B-G,** the left figures indicate measurements in individual patients

(blue dots) and black lines indicate the median score and IQR from all patients with available data. The right figures show the relation between the neurodevelopmental scores *versus* the chronological age using linear regression. A few, predominantly older patients , with an unexpectedly less severe neurocognitive phenotype (defined as having at least 2 of the following abilities: talk in simple words, attain head control, independent sitting, and/or walking with assistance) were excluded from the regression analysis (n=4 for GMFM-88 in panel B, and n=1 for BSID-III in panel C-G), which focused on individuals with classical sequelae of severe MCT8 deficiency (indicated with grey dashed lines in the left figure of each panel; n=40 for GMFM-88 in panel A, and n=27 for BSID-III in panel C-G). Linear regression was used to plot the trend (blue solid lines) and the 95% confidence intervals (blue dotted lines). Besides age, no other factors were considered in the models. Patients harboring the same genetic mutation are displayed in the same color: p.F230del (green), c.651-652+20del (blue), G564R (purple), p.V566X (pink), and R271H (orange). Unique mutations are colored in grey.

Figure 3 – Mean ± SEM (black lines) serum concentrations of thyroid stimulating hormone (TSH) (n=106) (panel **A**) and free T4 (n=106) (panel **B**). Blue dots represent measurements in individual patients and grey areas the normal range. Panel **C** presents the serum total T3 concentrations *versus* age (n=101). Panel **D** shows the available results on total T4 measurements during neonatal screening expressed in SDs (n=8). See **figure s9I** for TSH measurements during neonatal screening. Panel **E** shows the natural course of bodyweight change in patients with MCT8 deficiency. Blue dots represent available historical bodyweight measurements (n=300) in 86 untreated patients. Non-linear (third order) polynomial regression was used to plot the trend with its 95% error band. Similarly, panel **F** shows the natural course of body height. Accurate measurement of body height can be hampered by muscle contractions and involuntary movements. Panel **G** shows the resting heart rate by age

(n=64). Normal range in healthy children is derived from (17). Panel **H** shows the mean ± SEM diastolic and systolic blood pressure (n=47). The orange line represents the threshold for classification as elevated blood pressure and the red line the threshold of hypertension, as defined by the guidelines from the American Academy of Pediatrics (18) and the American College of Cardiology and American Heart Association (19). Panel **I** shows the mean ± SEM (black lines) occurrence of indicated features during 24h cardiac monitoring in 45 individuals. Serum concentration of sex hormone binding globulin (SHBG) (n=78) (panel **J**), creatinine (n=79) (panel **K**), and creatine kinase (n=79) (panel **L**) are expressed relative to the age-specific lower (panel **K**) or upper (panel **J** and **L**) limit of the normal range. Abbreviations: TSH, thyroid stimulating hormone; T4, thyroxine; T3, triiodothyronine; PACs, premature atrial contractions; PVCs premature ventricular contractions; CK, creatine kinase; SHBG, sex hormone binding globulin; LL, lower limit; UL, upper limit. The absolute mean values of all parameters are summarized in **table s4**.

Disease characteristics of MCT8 deficiency: an international, retrospective, multicentre cohort study

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Abstract

Background: Disordered thyroid hormone transport, due to mutations in monocarboxylate transporter 8 (MCT8; gene: *SLC16A2*), is characterized by intellectual and motor disability due to cerebral hypothyroidism and chronic peripheral thyrotoxicosis. Phenotypic characteristics and natural history of MCT8 deficiency have not been systematically evaluated.

Methods: In this international, multicentre, study, retrospective data (2003 to 2019) from patients with MCT8 deficiency followed in 47 centres, was analysed. Our primary objectives were to determine neurocognitive outcomes and overall survival. We also assessed clinical parameters, including anthropometric characteristics, biochemical markers and neuroimaging findings.

Results: 151 subjects with 73 different MCT8 (*SLC16A2*) mutations were included. 21·2% (32/151) of patients died, with main causes of mortality in these patients being pulmonary infection (18·8%) and sudden death (18·8%). The median overall survival was 35·0 (95%CI 8·3-61·7) years. Survival differed significantly between individuals who attained head control by the age of 1·5 years or not (log-rank test: p=0·0041; hazard ratio 3·46 95%CI 1·76-8·34). Patients who were underweight during early childhood (1-3 years of age) had an increased risk for death compared with patients who were not underweight at this age (HR 4·71, 95% CI 1·26-17·58, p=0·021). The limited motor and cognitive abilities of patients did not improve with age. T3 concentrations were elevated in 95·1% (96/101) and total T4 concentrations were reduced in 89·5% (94/105) of patients. 71·1% (59/83) patients were underweight (<-2SD). Cardiovascular abnormalities were frequent, with 53·2% (25/47) of patients exhibiting elevated systolic blood pressure, and 75·6% (34/45) of patients having premature atrial contractions and 31·3% (20/60) having resting tachycardia.

Interpretation Our description of characteristics of MCT8 deficiency in a large patient cohort reveals poor survival with a high prevalence of treatable underlying risk factors and provides knowledge which informs clinical management and future evaluation of therapies.

Funding Our study was funded by the Netherlands Organisation for Health Research and Development (project number 113303005; to WEV), and the Sherman Foundation (to WEV).

Research in context

Evidence before this study

Monocarboxylate transporter 8 (MCT8) deficiency is a rare genetic disorder with devastating consequences including intellectual and motor disability due to cerebral hypothyroidism and severe clinical sequelae secondary to chronic peripheral thyrotoxicosis. We searched Pubmed for studies published in English to January 1, 2020, using the search terms “MCT8 deficiency”, “Allan-Herndon-Dudley Syndrome”, “AHDS”, “natural history” and “life expectancy”. Prior to this study, given the rarity of the disorder, knowledge on the phenotypic characteristics, natural history and life expectancy of monocarboxylate transporter 8 (MCT8) deficiency was limited. Previous studies consisted of case reports, had small patient cohorts (<25 patients), and neglected the peripheral features of the disorder. Comprehensive and structured in-depth characterisation of the phenotype of MCT8 deficiency is urgently needed to accelerate early diagnosis and inform management, including the use of a new disease-modifying therapy.

Added value of this study

151 patients from 47 centres across the world were included in the largest study on MCT8 deficiency, to our knowledge. This is the first multicentre, international study to provide in-depth quantitative data on the natural history and life expectancy of patients with MCT8 deficiency. Our data report poor survival in this disorder, with 30% of patients dying in childhood. Having identified pulmonary infection and sudden death (our data suggests cardiac arrhythmia as underlying basis) as the major causes of mortality, timely intervention with Triac

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therapy may ameliorate the poor prognosis in this disease. Also, the identification of underweight being strongly linked to survival provides a direct target for clinical management. Our detailed description of key clinical features together with biochemical and radiological correlates constitutes a signature for the disorder which may facilitate its early diagnosis and discrimination of this entity from other developmental disorders. Our data will be used as natural history control data for an ongoing trial of with Triac in young children with MCT8 deficiency (NCT02396459). These data will also be important for future clinical trials investigating treatment options for MCT8 deficiency, such as gene therapy.

Implications of all the available evidence

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Systemic in-depth description of international natural history data will inform clinical management of patients with MCT8 deficiency. Our findings underscore the need for a multidisciplinary approach in the management and follow-up of patients with MCT8 deficiency. The current data indicate a unique combination of clinical presentation, biochemical markers and brain imaging features that will enhance early diagnosis. The low T4 concentrations measured in the neonatal screening indicates that current neonatal screening strategy holds potential to detect MCT8 deficiency. These observations hinting at the possibility of early diagnosis are particularly relevant in the context of Triac therapy recently reported, which has the potential to ameliorate the devastating course of the disorder if left untreated. In addition, robust natural history data can be used as controls in clinical trials for rare diseases in which accrual of placebo controls in group might not be feasible.

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Introduction

Thyroid hormones are crucial for normal physiological processes, particularly neurodevelopment, and regulation of basal metabolic rate, throughout life (1, 2). Intracellular bioavailability of thyroid hormones is governed by membrane transporter proteins that facilitate their cellular entry (3). Monocarboxylate transporter 8 (MCT8) is a specific thyroid hormone transporter that is crucial for transport of triiodothyronine (T3) and thyroxine (T4) in several tissues, including the brain (4-8). Mutations in the gene encoding MCT8 (*SLC16A2* on chromosome Xq13.2) cause MCT8 deficiency, also known as Allan-Herndon-Dudley syndrome (AHDS), a debilitating disorder with an estimated prevalence of 1 in 70 000 male individuals (9-11).

MCT8 deficiency is characterized by profound neurodevelopmental delay and a wide range of severe clinical sequelae secondary to chronic peripheral thyrotoxicosis which cannot be effectively treated with conventional (anti)thyroid drugs (3, 10, 11). In 2019, a clinical trial showed that treatment with triiodothyroacetic acid (Triac) ameliorates key features of peripheral thyrotoxicosis and might improve neurocognitive outcomes if treatment is commenced early in life (12).

Robust, comprehensive data regarding the phenotypic characteristics and natural history of patients with MCT8 deficiency are lacking, as the phenotype has only been recorded in single case reports or small case series with related patients [*e.g.* (13, 14)]. Furthermore, these reports used differing clinical methods precluding consistent assessments, and merely focused on the neurological phenotype, neglecting the peripheral clinical features of the disorder (3, 14). Data on survival and neurodevelopmental outcomes in this disorder are not known. The lack of consistent quantitative knowledge of the natural history and the

phenotypic spectrum of MCT8 deficiency hampers early diagnosis and uniform clinical management including the evaluation of a disease-modifying therapy.

Given the paucity of recorded data and with access to a large patient cohort via an international collaborative network for this rare disorder, we have sought to provide comprehensive and uniform phenotypic characterization of MCT8 deficiency using clinical, radiological, and biochemical data.

Methods

Study design and participants

This international study was initiated on 14 October 2014 by founding a consortium of centres where patients with MCT8 deficiency were followed before and after this date.

The key inclusion criterion was genetically confirmed MCT8 deficiency. Additionally, data on first-degree and second-degree male relatives with clinical MCT8 deficiency (when genetic testing was not available at that time) were included. There were no exclusion criteria. Our cohort consisted of patients, evaluated with a standardized protocol, who had been enrolled in the international, multicentre Triac Trial [NCT02060474, (12)] and patients who participated in the named patient program for Triac treatment and historical cases for whom Erasmus MC fulfilled a consultancy role following the first reports of MCT8 deficiency in 2004 (10, 11) (**figure s1**). The group of historical cases therefore contain patients who were alive and patients who were already deceased at time of enrollment. A subgroup of participants has been reported before with available individual case descriptions (n=47), or has been reported on aggregated level (n=46, (12)) (**figure s2**). For such patients, updated and exhaustive data were collected. For analysis of serum thyroid function tests, only patients

whose measurements were performed in the central laboratory of the Erasmus MC were considered to avoid inter-assay variation. For in-depth clinical and biochemical phenotyping only those patients were enrolled that either participated in the Triac Trial (12) or in the named patient program to ensure data had been captured by trained personnel and according to standard operating procedures.

Ethical considerations

This study conforms to the Declaration of Helsinki, Good Clinical Practice guidelines and was evaluated and approved by the appropriate local institutional review boards or ethics committees. However, for the retrospective analysis of existing datasets of patients in routine clinical care, the majority of centres did not require additional specific institutional review board approval. For other centres, studies were either ethically approved or the ethics committee provided a waiver for approval. Informed consent was obtained from the parents or legal representatives of all enrolled patients, unless the relevant institutional review board and/or local regulations had authorized the use of anonymised patient data without additional consent.

Procedures

An overview of study assessments and investigations is provided in **figure s1** and in the **Supplementary Methods**.

Outcomes

Our primary objective was to analyse the overall survival of patients with MCT8 deficiency and document causes of death. We also compared survival between patients who

did or did not attain full head control by the age of 1·5 years and between patients who were or were not already underweight by early childhood (between 1-3 years of age).

Other key objectives were to document neurocognitive function using uniform criteria and assess their relationship to biological age as a measure of disease progression and developmental outcome and to describe the occurrence of extra-neurological features.

Statistical analysis

We summarised continuous variables as mean and standard deviation (SD), or median and range. We established overall survival and compared patients with and without full head control and cases who were or were not underweight during early childhood with log-rank analysis. Survival was defined as the age at last date known alive. Hazard ratios were calculated using Cox regression models. Correlations between biological age and scores on different neuropsychological assessments were explored using linear regression. For these analyses, we excluded patients with a ~~n unexpectedly~~ less severe neurocognitive phenotype, defined as individuals that attained at least two of the following developmental milestones: talking in simple words, achieving head control, sitting independently, and/or walking with assistance. Higher developmental attainment in these patients is more likely to be due to the milder impact of the underlying MCT8 mutation than to the effect of aging. Assumptions for linear regression analyses were met. All statistical tests were two-sided, and p values of less than 0·05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism, version 6 (GraphPad, La Jolla, CA, USA).

Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and ~~had~~ final responsibility for the decision to submit for publication.

Results

In 47 centres, 151 patients of 22 different nationalities (8 ethnicities) were enrolled between October 14th 2014 and January 17th 2020 (**figure s1**), thereby including 50 percent of families reported thus far (**figure s2**). In 106 cases serum thyroid function tests had been measured in the central laboratory, and 86 had been checked according to standardised protocols for in-depth phenotyping at a median age of 4·8 years (interquartile range [IQR] 1·9-9·8, range 0·4-66·8) (**figure s1**).

The demographics and characteristics of the enrolled patients are summarised in **table s1**. In the 151 enrolled cases, 73 different underlying MCT8 mutations were identified, of which 36 had not been reported before (**figure s3**). A total of 17 mutations were identified in at least two unrelated families. All 35 missense mutations were located in the transmembrane helices (**figure s4**). The median age at diagnosis was 24·0 months (range: 0·0-744·0) (**figure 1A**), but the median age at onset of first symptoms was 4·0 months (range: 0·0-13·0) (**figure 1A**). Consequently, the median time to diagnosis was 18·0 months (IQR 7·8-63·0, range 0·0-738·0). The most frequently reported initial concerns that prompted medical evaluation were gross developmental delay (78·6%), hypotonia (39·8%), feeding problems (8·2%), and poor weight gain (7·1%) (**figure 1B**).

32 (21·2%) patients had died and the median age of their demise was 10·5 years (IQR 5·3-18·8, range 1·6-71·0). The main causes of death reported for these patients were

pulmonary infections (18·8%), sudden death (18·8%), and aspiration pneumonia (9·4%) (**figure 1C**). In 15 (46·9 %) of 32 deceased subjects the cause of death was unclear and postmortem examinations had not been performed. The median overall survival was 35·0 years (95% CI 8·3-61·7; **figure 1D**). The 10-, 18-, and 60-year survival probabilities were 85% (95% CI 78·0-92·0), 69·8% (58·2-80·3), and 34·8% (10·2-59·3), respectively. Patients not attaining full head control by the age of 1·5 years had an increased risk for death compared with patients who did attain head control (HR 3·46, 95% CI 1·76-8·34, p=0·0041; **figure 1E**). Patients who were underweight during early childhood (1-3 years of age) had an increased risk for death compared with patients who ~~were not underweight~~had a normal body weight by this age (HR 4·71, 95% CI 1·26-17·58, p=0·021, **figure 1F**).

The prevalence of specific neurological features in patients included in the in-depth phenotyped cohort (N=86, median age 4·8 years, IQR 1·9-9·8, range 0·4-66·8) is reported in **table 1** and **figure 2**, and neurological sequelae are summarized in **figure s5** and **figure s6**. All patients had moderate-to-severe intellectual disability with a severe delay in motor and language development (**table s2**). Only 6 (7·7%) patients achieved independent sitting and were less severely affected than the other patients (**figure 2A**). The median score on the Gross Motor Function Measure (GMFM)-88 (15) did not exceed 10% of the total score that should be obtained by healthy 4-year old children (**figure 2B**, **table s2**). Among 28 subjects that had been evaluated at a median age of 6·4 years (range 0·4-44·6) with the Bayley Scales of Infant Development (BSID)-III (16), the median developmental age was well-below 12 months on all tested sub-domains (**figure 2C-G**, **figure s7B-F**, **table s2**). Similar findings were obtained with the Vineland Adaptive Behavior Scale (VABS)-II (**figure s8, table s2).** The scores in any of the developmental domains did not correlate positively with age (e.g. motor skills: GMFM-88 B=-0·10 (95% CI, -0·29-0·09; p=0.29), BSID-III fine motor skills B= -0·11 (-0·23 - 0·01; p=0.072), and

BSID-III gross motor skills B= -0·04(-0·11- 0·02; p=0.17); **figure 2C-G**) and scores of patients with different age categories were not different (**table s2**)).

Pregnancy and delivery were unremarkable in the majority of cases, with most infants having ~~good Apgar scores,~~ normal birth weight ~~at term~~ and head circumference (**table 1**). At first presentation, most patients had global hypotonia with a pronounced head-lag on vertical suspension and upper trunk slipping through. Typically, by the end of the first year, dystonic posturing of the limbs and neck were noted. Exaggerated deep tendon reflexes were present in 80·3% (57/71) of cases, and 90·5% (67/74) of patients developed hypertonia in wrists, knees or heels with age attributed to dystonia and spasticity. Primitive reflexes remained present in 91·1% (51/56) cases, with a positive tonic neck reflex (81·0%) and glabellar sign (80·0%) being most prevalent, irrespective of patient age. Electro-encephalogram (EEG)-confirmed seizures were observed in 15 (23·1%) of 65 patients, and mostly involved generalized, absence-like episodes without a clear motor component.

MRI scans of the brain were available in 13 patients, performed at a median age of 8·0 months (range: 5·0-187·0), with 8 patients having at least one follow-up scan available (**table 1, figure s5**, **table s3**). The most consistent finding was a global delay in myelination, evidenced by diffuse residual hyperintense white matter in specific brain regions on T2-weighted images. Myelination improved with age, but had not fully normalized in the oldest patient (15 years) with available data. ~~Most cases showed diffuse cortical and subcortical atrophy with dilatation of the ventricles, widening of the subarachnoid spaces demonstrated by prominence of the supra- and infra-tentorial sulci.~~ ~~The~~se neuroradiological findings were supported by postmortem findings (see **supplementary results**).

Serum thyroid function tests were available in 106 treatment-naïve patients at a median age of 5·3 years (IQR 2·1-11·0, range 0·4-66·8). Serum TSH concentrations were within the normal range in 93 (88·6 %) of 105 patients (**figure 3A**). Serum free and total T4 concentrations were below the age-specific lower limit in 94 (88·7 %) of 106 and 94 (89·5%) of 105 patients, respectively (**figure 3B** and **figure s9A**). Mean serum T3 concentrations exceeded the age-specific upper limit in 96 (95·1%) of 101 patients (**figure 3C**), which resulted in a pronounced increase in the T3/T4 ratio (**figure s9B**). Reverse T3 (rT3) concentrations were decreased in 76 (90·5%) of 84 patients (**figure s9C**), with a concomitant increase in the T3/rT3 ratio (**figure s9D**). This endocrine signature was present regardless of age (**figure s9E-H**). In 3 out of 7 subjects TRH-stimulation tests showed an inadequate TSH response. In 7 (87·5%) out of 8 subjects in whom T4-based neonatal screening results were available, total T4 concentrations were below the 20th percentile (**figure 3D**), and in 5 out of 8 (60%) below the 10th percentile. By contrast, neonatal TSH concentrations were <15 mU/L in 8/8 (100%) of patients with available data (**figure s9I**). Serum total T4 concentrations were significantly less reduced in patients with less severe versus those with a severe neurocognitive phenotype (1·05±0·22 vs 0·71±0·18 times the age-specific lower limit of normal, p<0·0001) (**figure s10A)**. Serum T3 concentrations were not significantly different between these groups (1·46±0·23 vs 1·51±0·44 times the age-specific upper limit of normal, p=0·76)(**figure s10B**). Consequently, the T3/T4 ratio, a marker of thyroid hormone metabolism in peripheral tissues, was significantly lower in patients with a less severe phenotype (1·44±0·40 vs 2·27±0·91 times the age-specific upper limit of normal, p=0·019) (**figure s10C**).

The main findings of in-depth phenotyping of peripheral clinical features (n=86) are summarised in **table 2** and **table s4**. Body weight for age showed progressive deterioration over time, with 59 (71·1%) of 83 patients being severely underweight (<-2SD) (**figure 3E, table**

2). 27 (35·5%) of 76 patients were tube fed, although impaired swallowing function was reported in 55 (71·4%) of 77 patients. Body height for age also deteriorated with age (**figure 3F**). Delayed sexual maturation was present in 5 (26·3%) patients (**figure s11A-D**). Among patients over 8 years of age, bone mineral density (BMD) was below the 5th percentile, but bone turnover markers were generally within the low-normal range (**figure s12A-D**).

The mean resting heart rate was 110 (±20) beats per minute, with 20 (31·3%) of 64 patients exceeding the 90th percentile for age (**figure 3G**) (17). Systolic blood pressure exceeded the 90th percentile in 25 (53·2%) of 47 patients whereas the diastolic blood pressure exceeded the 90th percentile in 17 (36·2%) of 47 patients (**figure 3H**) (18, 19). Detailed cardiovascular assessment was available in 50 patients. At the time of evaluation, 47 (94%) of 50 patients reportedly had no cardiovascular abnormalities and were not receiving any treatment. 3 (6·0%) of 50 patients had second-degree atrioventricular block (Mobitz I: 1; Mobitz II: 2) and 6 (12·0%) of 50 patients had (incomplete) right bundle branch block. In addition, corrected QT interval (QTc) was above the 98th percentile in three out of 39 (7·7%) patients (**table 2**, **figure s13A**). Even though most patients were completely immobile, 24h ambulatory cardiac monitoring showed a high resting heart rate (103±13 beats per minute) with frequent episodes of tachycardia and premature atrial or ventricular contractions (**figure 3I, table s4**). One childhood patient had an episode of atrial fibrillation and another had non-sustained ventricular tachycardia (**table 2**). Cardiac echocardiography studies performed in 26 patients revealed dilated aortic root (>+2SD for age, range 2·0-3·4 SD) in 7 (26·9%) patients, and relatively small left ventricular wall dimensions (**figure s13B**).

Serum concentrations of sex hormone binding globulin were elevated in 69 (88·5%) of 78 patients (**figure 3J, figure s14**). Serum alanine aminotransferase, aspartate

aminotransferase, and gamma-glutamyl transferase concentrations were mildly elevated in a substantial proportion of patients (**table 2, figure s15D-F**), and two patients reportedly had an episode of hepatic dysfunction following a (viral) infection.

43 (84·3%) of 51 patients had low muscle mass. Creatinine concentrations in serum were within the low-normal range for age (**figure 3K, s14B**). Serum creatine kinase concentrations were mostly low-normal (**figure 3L, s14C**), with some exceptions in patients with recent seizures or severe dystonic episodes. Other biochemical tests are shown in table s4 and figure s15A-O.

Gastroesophageal reflux disease was present in 79·2% (38/48) of patients and this often required pharmacological intervention. Spontaneous gastrointestinal bleeding was reported in 2 patients and was the cause of death in one of them. Constipation was present in 62·3% (37/63) of patients. 29 (69·1%) of 42 patients had recurrent (pulmonary) infections (**table 2**).

Discussion

To our knowledge, this international, multicentre, retrospective study reports the quantitative evaluation of the disease characteristics of MCT8 deficiency, in the largest cohort of patients with this disorder. We have documented key clinical features together with biochemical and radiological correlates as well as outcomes in this rare but potentially treatable condition. Our findings will facilitate accurate diagnosis, guide management, and inform conduct of future therapeutic intervention trials.

A principal finding is that overall survival of patients with MCT8 deficiency is greatly diminished, with an overall median life expectancy of 35 years. Stratification of analyses

298 revealed that patients who attain full head control are more likely to survive longer than those
299 who do not. Accordingly, attaining full head control, as a marker of improved
300 neurodevelopment, could be a relevant endpoint for future therapeutic trials in MCT8
301 deficiency.

302 The most common cause of death was pneumonia, caused either by aspiration or by
303 infections. Aspiration, due to impaired swallowing function, is frequently observed in patients
304 with MCT8 deficiency, and could be mitigated by tube feeding. However, a substantial number
305 of patients that exhibited swallowing problems were not tube fed and thus remained at risk
306 for aspiration. With our study suggesting that being underweight is strongly linked to reduced
307 survival, tube feeding can prevent adverse clinical sequelae and potentially improve life
308 expectancy (20). The second major cause of mortality was sudden death. Although its
309 aetiology remains unclear, available data may suggest a cardiac cause, with the high
310 prevalence of premature atrial and ventricular contractions, which are uncommon in healthy
311 individuals especially in childhood (21-25). We also observed non-sustained ventricular
312 tachycardia and QTc prolongation in some patients, with both considered risk factors for
313 sudden cardiac death. Moreover, a substantial proportion of patients exhibited systolic
314 hypertension and/or tachycardia and had several echocardiographic and electrophysiological
315 cardiac changes that have been linked to these traits. As the vast majority (94·0%) of patients
316 reportedly had no history of cardiac problems, these cardiovascular abnormalities likely
317 remain clinically undiagnosed in this population. This observation calls for inclusion of
318 cardiovascular assessment in the management of this disorder. With loss of body weight and
319 many cardiovascular abnormalities being attributable to chronic thyrotoxicosis, reduction in
320 circulating T3 concentrations in patients could represent effective treatment for these aspects
321 of the disorder. Indeed, in a recent clinical trial, treatment with the thyroid hormone analogue

Triac efficiently reduced serum T3 concentrations and improved key clinical features such as loss of body weight and reversal of abnormal cardiovascular parameters in MCT8 deficiency (12).

The current study also identified several other clinical features that require treatment or close follow-up, of which gastro-esophageal reflux disease, scoliosis, hip luxation, and constipation have the highest prevalence. The presence of mildly elevated aminotransferases and the occurrence of transient hepatic failure in at least three reported cases following a viral infection [this report and (11)], suggests that drugs with hepatotoxic side effects (e.g. anti-epileptic drugs as frequently used in this population) should be used with extra caution.

Our comprehensive documentation of neurological sequelae in patients with MCT8 deficiency revealed that the combination of global hypotonia, hypertonia due to dystonia and spasticity and persistence of primitive reflexes was present in up to 90% of patients. Delayed myelination on MRI was consistent with other studies (14, 26, 27). Taken together, these clinical and neuroimaging characteristics may facilitate early diagnosis of MCT8 deficiency and in discriminating this entity from other neurodevelopmental disorders.

Our study highlights major delay in diagnosis of this disorder, with a minority of cases being identified in the first year of life. This is mainly attributable to the non-specific initial clinical features with lack of awareness of the specific characteristics of this disorder among clinicians. Having documented that circulating T3 concentrations are elevated in patients below one year of age, the combination of clinical and radiological features with measurement of serum T3 concentrations may constitute a key clue for early diagnosis. The low T4 concentrations measured in patients with MCT8 deficiency in the neonatal screening indicates the potential to diagnose MCT8 deficiency in newborns. This may engender debate on

whether modification of the current neonatal screening strategy is warranted. The importance of early diagnosis is supported by preclinical studies in which Triac completely prevented abnormal neurological development in animal models of MCT8 deficiency when administrated at birth (28). A future phase 2 clinical trial will investigate the effects of Triac on neurodevelopment, with treatment being commenced at a very young age (NCT02396459).

This study has limitations inherent to its retrospective design. In general, such study design is prone to collection of an incomplete dataset, possibly resulting in selection bias. Indeed, most assessments could not be carried out in all patients, resulting in missing data. The cause of this mostly reflected disease characteristics, such as a poor clinical condition of patients, their inability to follow instructions and dystonic posturing that hampered investigations for which patients needed proper positioning. Therefore, some conclusions are based on a limited number of observations. Yet, calculated prevalence rates for many clinical features were similar to those observed in smaller cohort studies and through analysis of existing literature (14). It was also not possible to obtain uniform long-term follow-up data. However, MCT8 deficiency is a rare disorder with surviving patients being located throughout the world such that retrospective analysis of available clinical data was the most suitable way of increasing our understanding of this disorder. In the majority of cases data had been collected uniformly during baseline assessment of patients whether participating in the Triac Trial or in named patient treatment programs, providing an unique opportunity for systematic cross-sectional evaluation of key clinical outcomes. Should MCT8 deficiency result in an increased rate of miscarriage this will likely remain unascertained, resulting in survivor bias. Although selection bias cannot be excluded, probably because not all newly diagnosed cases are brought to our attention and patients who die before the diagnosis has been established are being missed, our study included a substantial proportion of currently diagnosed patients.

With advent of Triac as possible disease-modifying therapy, a future, prospective cohort study of the natural history of the disorder is unlikely to be possible.

In summary, this study provides a comprehensive and structured in-depth characterisation of the phenotype of MCT8 deficiency. The current study first reports poor survival in this disorder, with 30% of patients dying in childhood. Having identified pulmonary infection and sudden death (our data suggests cardiac arrhythmia as underlying basis) as the major causes of mortality, timely intervention with Triac therapy may ameliorate the poor prognosis in this disease. Furthermore, our finding that survival is particularly poor in patients with impaired neurological development (head control) or who are underweight, provides a basis for therapeutic intervention targeted at this subgroup. Our findings underscore the need for a multidisciplinary approach in the management and follow-up of patients with MCT8 deficiency. In addition, our observations represent an unique, quantitative dataset of the characteristics of this disorder which may serve as a historical control for future interventional studies in this rare disorder, for which a biological control group is often deemed not feasible. Accordingly, we suggest that this study enhances our understanding of the clinical sequelae and longterm outcome of MCT8 deficiency and also facilitates the diagnosis and management of this disorder.

Contributors

SG, FSvG, WEV, IFMdC, and MD designed the study, acquired and analysed the results and drafted and approved the manuscript. All other authors contributed to the acquisition, analysis, and interpretation of data, and approved the manuscript.

Declaration of Interest

~~Dr. Visser~~WEV reports grants from Netherlands Organisation for Health Research and Development and from Sherman Foundation during the conduct of the study. ~~Drs~~ ~~Visser~~WEV, ~~Groeneweg~~SG, ~~Van Geest~~FSvG report other from Rare Thyroid Therapeutics, outside the submitted work. ~~Dr. Craiu~~DC reports grants from BioMarine, grants from UCB, grants from A&D pharma, outside the submitted work. ~~Dr. de Wit~~MCYdW reports other from Hoffmann-La Roche Ltd, other from Ionis, outside the submitted work.~~The Erasmus Medical Centre (Rotterdam, Netherlands), which employs SG, FSvG, IMvB, MD, MMvdK, CAU, MCYdW, and WEV, might receive royalties from Rare Thyroid Therapeutics (the manufacturer of Triac) in the future, dependent on any future commercialisation. None of the authors will benefit personally from any royalties. Rare Thyroid Therapeutics had no influence on the conduct or analysis of this study~~. All other authors declare no competing interests.

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Data sharing

Because of the rarity of MCT8 deficiency, individual participant data beyond that reported here will not be shared, to safeguard patient privacy.

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Legends to the Figures

Figure 1 – Panel **A** graphically presents the mean ± SEM (black lines) age at onset of symptoms and the age at time of diagnosis. Grey dots represent measurements in individual patients. Panel **B** shows the most commonly reported features that prompted parents to seek medical attention. Panel **C** shows the causes of death in patient with MCT8 deficiency based on the available information derived from the 32 patients in our cohort who died at a median age of 10·5 years (IQR 5·3-18·8, range 1·6-71·0). HR: hazard ratio, calculated using Cox regression models. Panel **D** shows the overall survival based on age at last follow-up (Kaplan-Meier estimates). Panel **E** shows the Kaplan-Meier estimates of MCT8-specific survival in patients who attained head control (red line) by the age of 1·5 years *versus* those who did not (blue line) and panel **F** those in patients with underweight (blue line) *versus* ~~without~~ normal body weight (red line) ~~underweight~~ in early childhood (1-3 years of age). Underweight was defined as a body weight for age z score <-2SDs (or below the fifth percentile), following the definition of the World Health Organization. Please note, since potential confounding factors could not be accounted for, a causal relationship on survival cannot be inferred from the applied stratification variables.

Figure 2 – Panel **A** shows the prevalence of clinical, radiological and developmental key features in MCT8 deficiency. Bars indicate the proportion of patients presenting the indicated feature at first presentation. Panel **B** represents the gross motor function development in patients with MCT8 deficiency measured by the Gross Motor Function Measure (GMFM)-88 (15). A 100% score indicates the level of development that is achieved by a healthy 4-year old child. Panel **C** shows cognition, panel **D** receptive language, panel **E** expressive language, panel **F** fine motor skills and panel **G** gross motor skills, measured by the respective sub-domains of the Bayley Scales of Infant Development (BSID)-III (16). Scores are expressed as developmental

age in months. In panels **B-G,** the left figures indicate measurements in individual patients (blue dots) and black lines indicate the median score and IQR from all patients with available data. The right figures show the relation between the neurodevelopmental scores *versus* the chronological age using linear regression. A few, predominantly older patients , with an unexpectedly less severe neurocognitive phenotype (defined as having at least 2 of the following abilities: talk in simple words, attain head control, independent sitting, and/or walking with assistance) were excluded from the regression analysis (n=4 for GMFM-88 in panel B, and n=1 for BSID-III in panel C-G), which focused on individuals with classical sequelae of severe MCT8 deficiency (indicated with grey dashed lines in the left figure of each panel ; n=40 for GMFM-88 in panel A, and n=27 for BSID-III in panel C-G) Linear regression was used to plot the trend (blue solid lines) and the 95% confidence intervals (blue dotted lines). Besides age, no other factors were considered in the models. Patients harboring the same genetic mutation are displayed in the same color: p.F230del (green), c.651-652+20del (blue), G564R (purple), p.~~A565fs566X~~ V566X (pink), and R271H (orange). Unique mutations are colored in grey.

Figure 3 – Mean ± SEM (black lines) serum concentrations of thyroid stimulating hormone (TSH) (n=106) (panel **A**) and free T4 (n=106) (panel **B**). Blue dots represent measurements in individual patients and grey areas the normal range. Panel **C** presents the serum total T3 concentrations *versus* age (n=101). Panel **D** shows the available results on total T4 measurements during neonatal screening expressed in SDs (n=8). See **figure s9I** for TSH measurements during neonatal screening. Panel **E** shows the natural course of bodyweight change in patients with MCT8 deficiency. Blue dots represent available historical bodyweight measurements (n=300) in 86 untreated patients. Non-linear (third order) polynomial regression was used to plot the trend with its 95% error band. Similarly, panel **F** shows the

natural course of body height. Accurate measurement of body height can be hampered by muscle contractions and involuntary movements. Panel **G** shows the resting heart rate by age (n=64). Normal range in healthy children is derived from (17). Panel **H** shows the mean ± SEM diastolic and systolic blood pressure (n=47). The orange line represents the threshold for classification as elevated blood pressure and the red line the threshold of hypertension, as defined by the guidelines from the American Academy of Pediatrics (18) and the American College of Cardiology and American Heart Association (19). Panel **I** shows the mean ± SEM (black lines) occurrence of indicated features during 24h cardiac monitoring in 45 individuals. Serum concentration of sex hormone binding globulin (SHBG) (n=78) (panel **J**), creatinine (n=79) (panel **K**), and creatine kinase (n=79) (panel **L**) are expressed relative to the age-specific lower (panel **K**) or upper (panel **J** and **L**) limit of the normal range. Abbreviations: TSH, thyroid stimulating hormone; T4, thyroxine; T3, triiodothyronine; PACs, premature atrial contractions; PVCs premature ventricular contractions; CK, creatine kinase; SHBG, sex hormone binding globulin; LL, lower limit; UL, upper limit. The absolute mean values of all parameters are summarized in **table s4**.

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Table 1. In depth phenotyping of neurodevelopmental features	
	N=86
Age at assessment (years)	4·8 (0·44-66·8)
Perinatal features	
Pregnancy duration (weeks)	40·0 (32·0-42·3)
Apgar scores >8 after 5 min (n=16)	15 (93·8)
Term birth weight (grams)	3584 (±517)
Microcephaly (<3th centile) at birth (n=11)	2 (18·2)
Neurological examination	
Hypotonia (n=72)	72 (100%)
Primitive reflexes (>1 present) (n=56)	51 (91·1%)
Tonic neck reflex (n=21)	17 (81·0%)
Glabellar sign (n=55)	44 (80·0%)
Startle response (n=25)	17 (68·0%)
Scoliosis (>8 years) (n=17)	15 (88·2%)
Muscle hypoplasia (n=51)	43 (84·3%)
Dystonia (n=69)	57 (82·6%)
Spasticity (n=71)	57 (80·3%)
Urinary / faecal incontinence (>4 years) (n=41)	33 (80·5%)
Feeding problems (n=77)	55 (71·4%)
Hip dislocation (>8 years, n=15)	10 (66·7%)
Plantar extension response (Babinski sign, n=57)	38 (66·7%)
Delayed evoked potentials (<6 months, n=6)*	3 (50·0%)
Sleep problems (n=51)	20 (39·2%)
Tube feeding (n=76)	27 (35·5%)
Strabismus (n=54)	19 (35·2%)
Microcephaly (<3th centile) (n=59)	19 (32·2%)
Nystagmus (n=49)	13 (26·5%)
Extrapyramidal signs (other) (n=28)	7 (25·0%)
Seizures (EEG proven) (n=65)	15 (23·1%)
Apneusis (n=32)	7 (21·9%)
Abnormal hearing (n=44)	1 (2·3%)
Delayed evoked potentials (>1 year, n=3)	0 (0·0%)
Development	
Head control (n=77)	19 (24·7%)
Speech (at least 1 word) (n=76)	5 (6·6%)
Independent sitting (n=78)	6 (7·7%)
Independent walking (n=77)	4 (5·2%)
MRI/MRS characteristics*	
Normal global anatomy (n=13)	13 (100%)
Delayed myelination (n=13)	13 (100%)
Reduced cerebral white matter volume (n=13)	13 (100%)
Periventricular white matter lesions (n=10)	10 (100%)
Prominent supratentorial ventricular system (n=13)	13 (100%)
Prominent peripheral liquor spaces (n=13)	13 (100%)
Low NAA peak (n=7)	6 (85·7%)
High choline peak (n=7)	6 (85·7%)

Data are median (range), n (%), or mean (±SD). Systematic deep phenotyping of neurological phenotype in 86 eligible patients. Median age at last available MRI scan: 18·0 months, range 5·0-187·0); MRS was available in 7 cases. Details are provided in **table s2**.

* In particular the brainstem-evoked response audiometry was abnormal in children < 1 year of age and showed prolongation of the I-V interval.

1

Table 2. In depth phenotyping of peripheral features	
Characteristic	**N=106**
Serum thyroid function tests	
Age at measurement (years)	5·3 (0·4-66·8)
Elevated T3 concentrations (n=101)	96 (95·1%)
Reduced free T4 concentrations (n=106)	94 (88·7%)
Deep phenotyping	**N=86**
Age at assessment (years)	4·8 (0·44-66·8)
Biochemical measurements *	
Elevated sex hormone binding globulin (n=78)	69 (88·5%)
Elevated alanine aminotransferase (n=65)	30 (46·2%)
Reduced creatinine (n=79)	22 (27·8%)
Elevated lactate (n=11)	3 (27·3%)
Reduced total cholesterol (n=65)	12 (18·5%)
Elevated aspartate aminotransferase (n=56)	11 (19·6%)
Elevated creatine kinase (n=79)	3 (3·8%)
Clinical features	
Low bone mineral density (>8 years, n=5)	5 (100%)
Hypotrophic musculature (n=51)	43 (84·3%)
Gastro-esophageal reflux disease (n=48)	38 (79·2%)
Premature atrial complexes (n=45)	34 (75·6%)
Recurrent (pulmonary) infections (n=42)	29 (69·0%)
Underweight (<-2 SD, n=83)	59 (71·1%)
Constipation (n=63)	37 (58·7%)
Elevated systolic blood pressure ¶ (n=47)	25 (53·2%)
Increased perspiration (n=60)	29 (48·3%)
Short stature (<-2 SD, n=67)	27 (40·3%)
Premature ventricular complexes (n=45)	19 (42·2%)
Tachycardia in rest † (n=64)	20 (31·3%)
Aortic root dilatation (n=26)	7 (26·9%)
Elevated diastolic blood pressure ¶ (n=47)	17 (36·2%)
Delayed sexual maturation (>8 years, n=19)	5 (26·3%)
Cardiac conduction abnormalities ‡ (n=50)	9 (18·0%)
Cryptorchidism (n=49)	9 (18·4%)
Prolonged QTc interval (39)	3 (7·7%)
Supraventricular tachycardia (n=48)	2 (4·2%)
(Non-sustained) ventricular tachycardia (n=48)	2 (4·2%)
Atrial fibrillation (n=48)	1 (2·1%)

Data are median (range), or n (%). Systematic deep phenotyping of the peripheral phenotype. Please note that most parameters have not been captured in all patients. All absolute and relative values are provided in **table s4**.

* Reduced and elevated indicated concentrations below or above the normal range (2.5-97.5 centile in the healthy population).

† Tachycardia was defined as a resting heart rate above the 90th percentile for the corresponding age, with cut-offs described by Fleming and colleagues **(17)**.

‡Three out of 50 patients (5·5%) had a second degree atrioventricular block (Mobitz I:1; Mobitz II:2) and 6 out of 50 patients (12·0%) had an (incomplete) right bundle branch block and 1 patient (2·0%) had a left posterior hemiblock.

¶ Elevated systolic and diastolic blood pressure were defined using the guidelines from the American Academy of Pediatrics **(18)** and the American College of Cardiology and American Heart Association **(19)**.

Figure 1

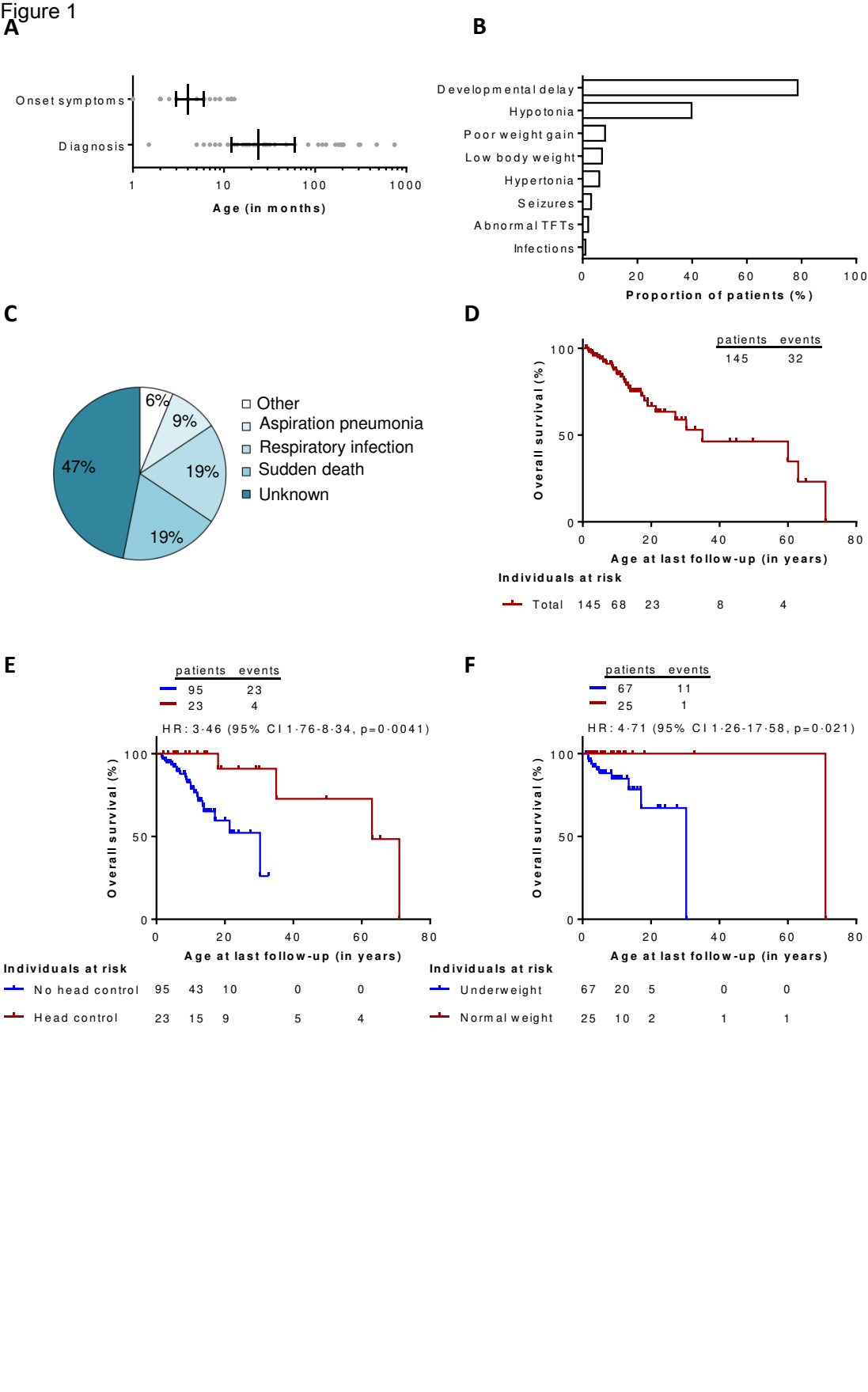

A
Onset symptoms
Diagnosis
1
10
100
1000
Age (in months)
B
Developmental delay
Hypotonia
Poor weight gain
Low body weight
Hypertonia
Seizures
Abnormal TFTs
Infections
0
20
40
60
80
100
Proportion of patients (%)
C
6%
9%
19%
19%
47%
Other
Aspiration pneumonia
Respiratory infection
Sudden death
Unknown
D
patients events
145 32
Overall survival (%)
100
50
0
0
20
40
60
80
Age at last follow-up (in years)
Individuals at risk
Total 145 68 23 8 4
E
patients events
95 23
23 4
HR: 3·46 (95% CI 1·76-8·34, p=0·0041)
Overall survival (%)
100
50
0
0
20
40
60
80
Age at last follow-up (in years)
Individuals at risk
No head control 95 43 10 0 0
Head control 23 15 9 5 4
F
patients events
67 11
25 1
HR: 4·71 (95% CI 1·26-17·58, p=0·021)
Overall survival (%)
100
50
0
0
20
40
60
80
Age at last follow-up (in years)
Individuals at risk
Underweight 67 20 5 0 0
Normal weight 25 10 2 1 1

Figure 2

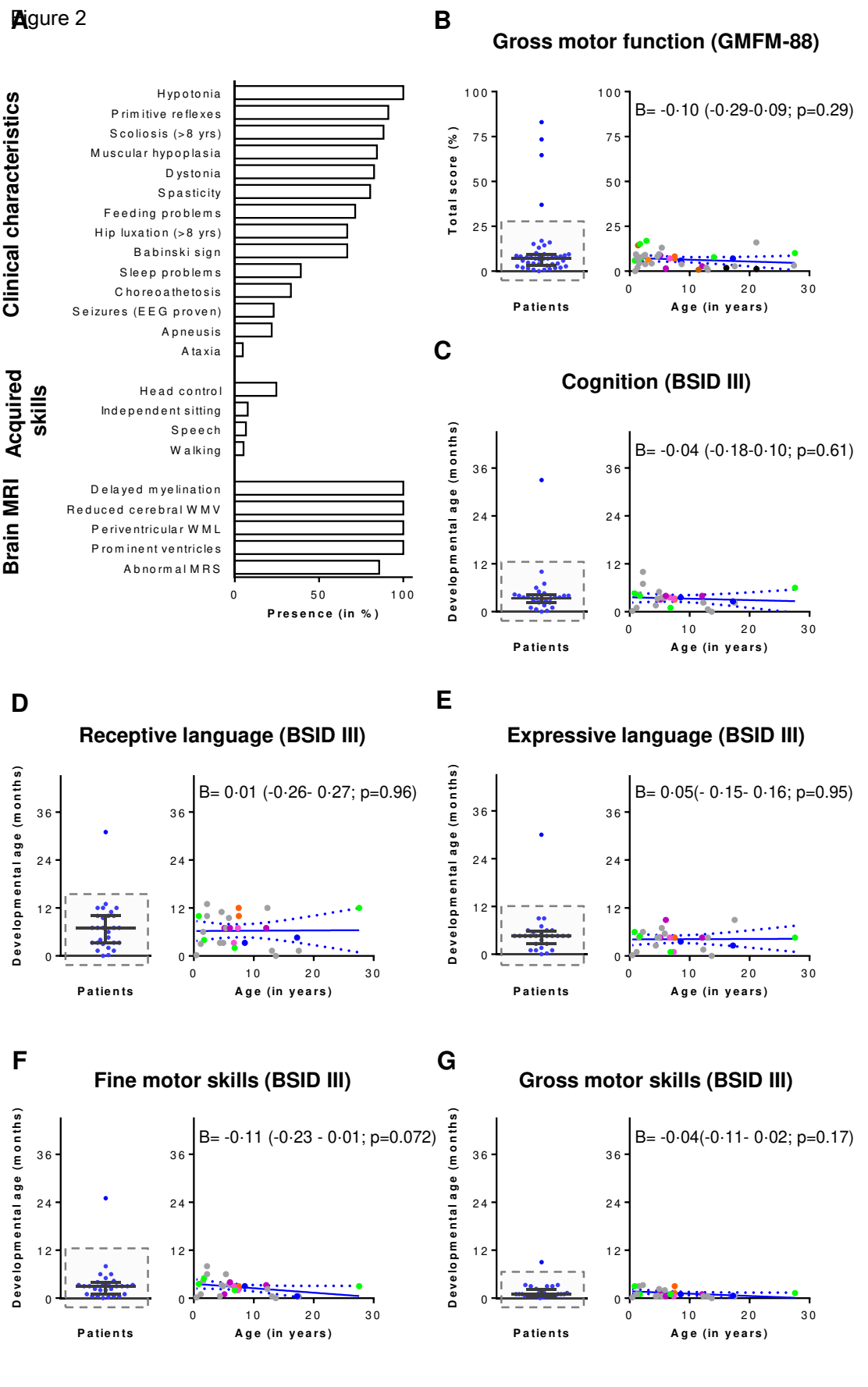
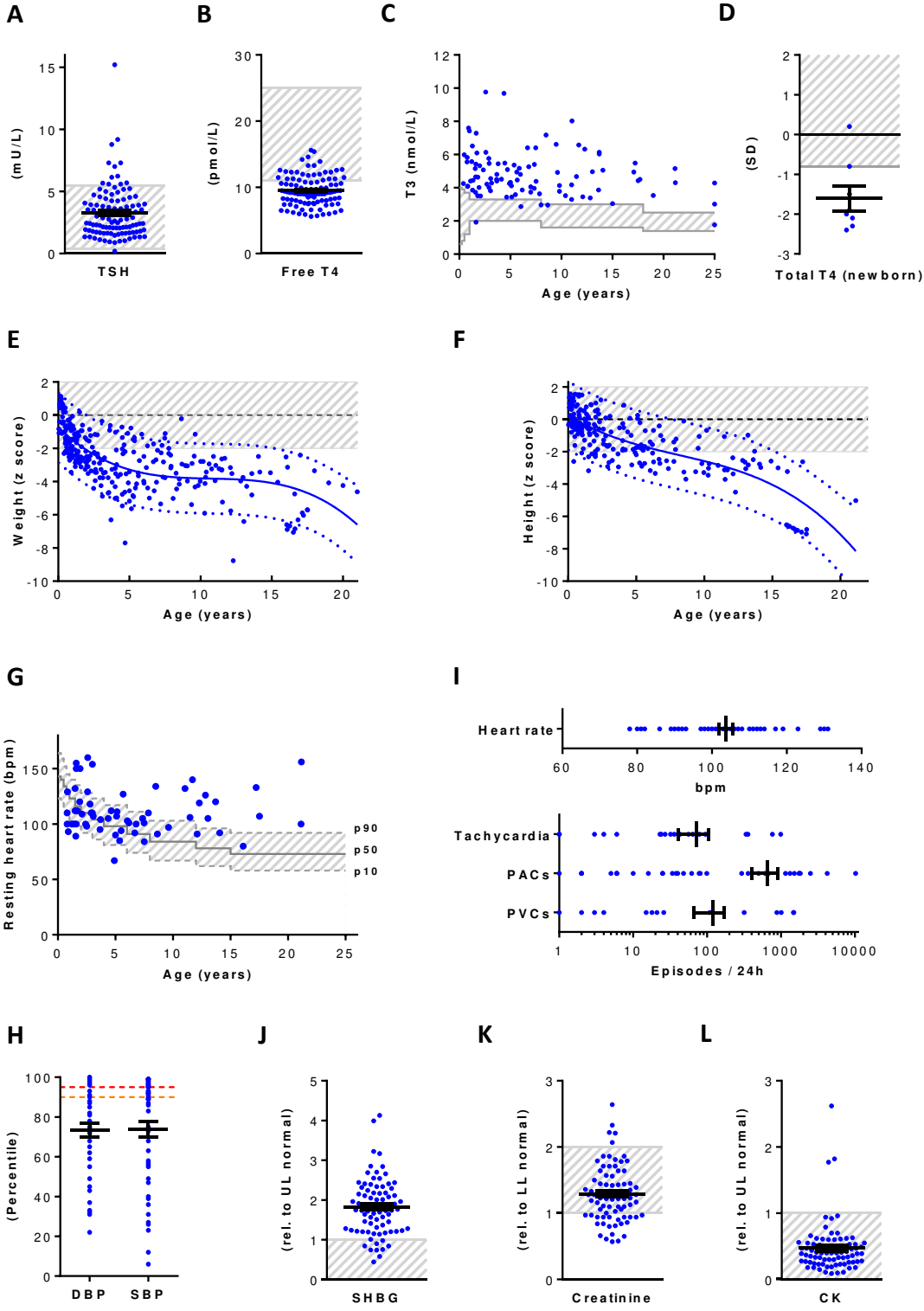

A
Clinical characteristics
Hypotonia
Primitive reflexes
Scoliosis (>8 yrs)
Muscular hypoplasia
Dystonia
Spasticity
Feeding problems
Hip luxation (>8 yrs)
Babinski sign
Sleep problems
Choreoathetosis
Seizures (EEG proven)
Apneusis
Ataxia
Acquired skills
Head control
Independent sitting
Speech
Walking
Brain MRI
Delayed myelination
Reduced cerebral WMV
Periventricular WML
Prominent ventricles
Abnormal MRS
0
50
100
Presence (in %)
B
Gross motor function (GMFM-88)
Total score (%)
B= -0·10 (-0·29-0·09; p=0.29)
Patients
Age (in years)
C
Cognition (BSID III)
Developmental age (months)
B= -0·04 (-0·18-0·10; p=0.61)
D
Receptive language (BSID III)
B= 0·01 (-0·26- 0·27; p=0.96)
E
Expressive language (BSID III)
B= 0·05(- 0·15- 0·16; p=0.95)
F
Fine motor skills (BSID III)
B= -0·11 (-0·23 - 0·01; p=0.072)
G
Gross motor skills (BSID III)
B= -0·04(-0·11- 0·02; p=0.17)

Figure 3


A
B
C
D
(mU/L)
TSH
(pmol/L)
Free T4
T3 (nmol/L)
Age (years)
(SD)
Total T4 (newborn)
E
F
Weight (z score)
Age (years)
Height (z score)
Age (years)
G
I
Resting heart rate (bpm)
p90
p50
p10
Age (years)
Heart rate
bpm
Tachycardia
PACs
PVCs
Episodes / 24h
H
J
K
L
(Percentile)
DBP
SBP
(rel. to UL normal)
SHBG
(rel. to LL normal)
Creatinine
(rel. to UL normal)
CK

Supplemental File with revisions highlighted (word document)

Supplemental File with revisions highlighted (PDF document)

Supplemental File clean (PDF document)

Supplemental File clean (word document)